SHORT RESUME/SYNOPSIS OF Ph.D. TOPIC

Entitled

"BIOSYNTHESIS, CHARACTERIZATION AND EFFECT OF HONEY MEDIATED ZINC OXIDE NANOPARTICLES ON MORPHOLOGICAL ASPECTS OF *Labeo rohita* (Hamilton, 1822)"

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1. A BREIF RESUME OF PROPOSED WORK/ PROBLEM:

Nanotechnology is an advancing field with transformative applications across various disciplines, including aquaculture. Among the numerous nanoparticles, zinc oxide nanoparticles (ZnO NPs) have gained significant attention due to their exceptional antimicrobial properties, biocompatibility, and multifunctional applications. However, traditional methods of synthesizing ZnO NPs often involve hazardous chemicals and energy-intensive processes, raising environmental concerns. Green synthesis using natural reducing agents, such as honey, provides an eco-friendly alternative, aligning with the global drive for sustainable and environmentally safe practices.

Honey-mediated synthesis of ZnO NPs is a promising green nanotechnology approach due to honey's natural abundance of phytochemicals, antioxidants, and reducing agents, which facilitate nanoparticle formation. This research focuses on utilizing honey as a green reducing agent to biosynthesize ZnO NPs, characterize their physicochemical properties, and evaluate their impact on the morphology of *Labeo rohita*, a key freshwater aquaculture species.

Aquaculture is a critical food production sector globally, and in India, *Labeo rohita* is one of the most widely cultured fish species. Despite its importance, aquaculture faces challenges such as disease outbreaks, environmental stressors, and a lack of innovative, sustainable solutions. The proposed research aims to explore the potential of honey-mediated ZnO NPs as a tool to improve fish health and enhance aquaculture productivity. In regard to this, the study focuses on developing eco-friendly zinc oxide nanoparticles (ZnO NPs) using honey as a reducing and stabilizing agent. The synthesized nanoparticles will be evaluated for their physicochemical properties and tested for their effects on the morphological aspects of *Labeo rohita*, a freshwater fish of significant aqua cultural importance including:

- Honey-mediated ZnO NPs will be synthesized and characterized.
- Evaluating Antimicrobial Activity of ZnO NPs against fish pathogens to determine their disease-preventive potential.
- Morphometric Analysis of *Labeo rohita* its body length, weight, and condition factor will be assessed to understand growth and development trends.
- Morphological Analysis and Evaluate how ZnO NPs influence the physical and structural morphology of *Labeo rohita*.
- Growth pattern and fin morphological analysis of *Labeo rohita.*
- To evaluate the potential benefits of ZnO-NPs in *Labeo rohita.*
- Biochemical, Hematological and Histological analysis of *Labeo rohita.*

2. INTRODUCTION TO PROPOSED RESEARCH TOPIC:

Nanotechnology is an innovative and transformative field with applications across diverse sectors, including medicine, agriculture, and aquaculture. Among the various nanomaterials, zinc oxide nanoparticles (ZnO NPs) have emerged as highly valuable due to their unique properties, such as antimicrobial activity, antioxidant potential, and environmental compatibility. Synthesis of nanoparticles can be categorized into top down or bottom-up approaches (C. J. Murphy, 2002). Top-down approach involves a process of breaking down of large structures to create small structures. However, the conventional methods for synthesizing ZnO NPs often rely on chemical processes that can be harmful to the environment, involve toxic chemicals, and produce hazardous byproducts. To overcome these challenges, the use of green synthesis techniques, which employ biological materials like plant extracts, microorganisms, or natural products, has gained attention. Zinc oxide is strongly included in this field, as it leads to a combination of oxides of metal, which is characterized by its susceptibility to photo stimulation and high photo oxidation as compared to many chemical and biological compounds (Zhang H et al.,2011).

Honey is one such natural product that offers significant potential for green nanoparticle synthesis. It is rich in bioactive compounds, including flavonoids, phenols, and enzymes, which act as reducing and stabilizing agents during nanoparticle formation. Natural honey is a sticky

and viscous solution, depending on its water content (T. Eteraf-Oskouei and M. Najafi, 2013). It also has the ability to absorb and hold moisture from the environment. Normal honey with a water content of 18.8% or less will absorb moisture from air of a relative humidity of above 60% (P. B. Olaitan et al., 2007).These products have been known for their medicinal and health-promoting properties since ancient times. The use of honey as a medium for biosynthesis is not only eco-friendly but also produces nanoparticles with enhanced biocompatibility and stability, making them suitable for applications in sensitive environments such as aquaculture.

In aquaculture, improving the health, immunity, and growth of fish is a major goal to meet the rising demand for sustainable fish farming practices. *Labeo rohita*, commonly known as rohu, is a commercially important freshwater fish widely cultivated in Asia. However, it is susceptible to environmental stress, pathogens, and diseases, which can negatively impact its productivity and survival. ZnO NPs, synthesized using honey, hold promise as a sustainable solution to these challenges. These nanoparticles can enhance fish health by acting as growth promoters, reducing pathogen loads, and improving water quality.

The proposed study aims to investigate the biosynthesis of ZnO NPs using honey, characterize their physicochemical properties, and evaluate their effects on the morphological aspects of *Labeo rohita*. Morphological parameters, including body structure, scales, fins, and swimming behavior, will be assessed to understand the potential benefits and safety of ZnO NPs in aquaculture. Additionally, the study will contribute to understanding the environmental impact of green-synthesized nanoparticles and establish guidelines for their safe application in fish farming systems.

This research is significant as it combines eco-friendly nanotechnology with sustainable aquaculture practices, addressing both environmental and economic challenges. By exploring the potential of honey-mediated ZnO NPs, the study aims to provide insights into their role in improving fish health, reducing disease prevalence, and enhancing the overall productivity of aquaculture systems. The findings are expected to benefit the aquaculture industry by promoting innovative, safe, and sustainable methods to meet the growing global demand for fish and aquatic products.

3. SURVEY OR REVIEW OF LITERARURE AND DEVELOPMENT IN THE RELEVANT SUBJECT :

The synthesis and application of nanoparticles, particularly zinc oxide nanoparticles (ZnO NPs), have emerged as a promising field of research in recent years. Green synthesis methods, which utilize natural resources like plant extracts, microorganisms, and honey, are particularly gaining attention due to their eco-friendly and sustainable nature. This review summarizes significant contributions in the field, with a focus on the biosynthesis, characterization, and effects of ZnO NPs on aquatic organisms, specifically *Labeo rohita*.

The use of biological agents for nanoparticle synthesis offers a sustainable and environmentally friendly approach. Honey has been identified as a natural medium for ZnO NP biosynthesis due to its rich phytochemical content, which includes flavonoids, phenolic acids, and enzymes. These compounds serve as reducing and stabilizing agents during the synthesis process.

Ahmed et al. (2016) reported the use of honey in synthesizing ZnO NPs with excellent antimicrobial and antioxidant properties.

Salem et al. (2018) demonstrated that honey-derived ZnO NPs exhibit higher biocompatibility and reduced toxicity compared to chemically synthesized nanoparticles.

Khalid et al. (2017) demonstrated that honey-mediated ZnO NPs have superior antimicrobial and antioxidant properties compared to chemically synthesized nanoparticles.

Wang et al. (2017) employed SEM, TEM, and XRD to analyze the crystalline structure and surface morphology of ZnO NPs, confirming their nanoscale size and unique physicochemical properties.

Ali et al. (2021) found that dietary supplementation with ZnO NPs improved growth performance, immune response, and oxidative stress resistance in freshwater fish species.

Ramesh et al. (2019) investigated the effects of ZnO NPs on the morphological features of *Labeo rohita*, observing improvements in scale integrity and fin health at optimal concentrations.

Rai et al. (2021) observed that ZnO NPs, when used at optimal concentrations, enhanced the overall health and resistance of fish against environmental stressors.

4. OBJECTIVE OF THE RESEARCHAND PROPOSED HYPOTHESIS :

The objective of the study is to:

- To biosynthesize zinc oxide nanoparticles (ZnO NPs) using honey as a natural reducing and capping agent.
- To analyze the size, shape, and morphology of ZnO NPs synthesized with honey.
- To evaluate the antimicrobial activity of the synthesized ZnO NPs.
- Collection of *Labeo rohita* from Latur city market.
- Evaluate the water quality of the aquarium.
- Study the Morphometric analysis of *Labeo rohita.*
- To evaluate the impact of honey-mediated ZnO nanoparticles on the growth and development of *Labeo rohita*.
- To investigate the physiological, hematological, biochemical, histological, and morphological effects of ZnO NPs on the freshwater fish *Labeo rohita*.

5. METHODOLOGY TO BE USED AND ADOPTED :

The study involves the biosynthesis of zinc oxide (ZnO) nanoparticles using natural honey as a reducing and stabilizing agent. Initially, an aqueous solution of zinc nitrate will be prepared as the precursor. Honey will be added dropwise to the solution under constant stirring at room temperature. The reaction mixture will be heated to optimize the formation of ZnO nanoparticles, and the resulting colloid will be centrifuged to separate the nanoparticles. The synthesized nanoparticles will be washed with distilled water and dried at a controlled temperature to obtain a fine powder.

The characterization of ZnO nanoparticles will be carried out using techniques like UV-Vis spectroscopy, X-ray diffraction (XRD), and scanning electron microscopy (SEM) to confirm their size, structure, and morphology.

For the assessment of morphological impact, healthy fingerlings of *Labeo rohita* will be selected and acclimatized to laboratory conditions. The fish will be divided into control and experimental groups, where the experimental groups will be exposed to different concentrations of ZnO nanoparticles in the water for a specific duration. Water quality parameters will be monitored throughout the experiment.

Post-exposure, fish will be observed for behavioral changes and morphological abnormalities. Histopathological analysis will be performed to evaluate nanoparticle-induced alterations. Data obtained will be statistically analyzed to identify significant differences between treated and control groups, providing insights into the ecological risks of ZnO nanoparticles.

The study on the biosynthesis of honey-mediated zinc oxide nanoparticles (ZnO NPs) and their impact on the morphology of *Labeo rohita* hold significant importance in the fields of nanotechnology, aquaculture, and environmental sustainability.

6. IMPORTANCE OF STUDY:

International Status- Nanotechnology has become a cornerstone of scientific innovation globally, with ZnO NPs gaining prominence due to their antimicrobial properties, eco-friendliness, and broad applicability. Green synthesis using honey offers an alternative to conventional chemical synthesis, aligning with global goals for sustainable development and environmental protection. Studies on ZnO NPs are particularly relevant in aquaculture, where disease management and water quality enhancement are critical challenges. This study will provide critical insights into the safe and effective application of ZnO NPs in fish farming, which can influence international aquaculture practices. Furthermore, the focus on *Labeo rohita*, a freshwater fish, expands the knowledge base to species relevant in developing regions, making the study globally significant.

National Status- India is the second-largest producer of fish worldwide, with aquaculture contributing significantly to national food security and rural livelihoods. *Labeo rohita* (rohu) is a major aquaculture species in the country, and its health and productivity are vital to the economy. Disease outbreaks and environmental concerns, such as water contamination, are key challenges in Indian aquaculture.

This study aligns with India's focus on promoting sustainable and innovative practices in aquaculture. Honey-mediated ZnO NPs, with their antimicrobial properties, offer a promising solution for disease management without relying on harmful chemicals or antibiotics. Additionally, the use of honey for green synthesis promotes the utilization of natural, abundant resources in India.

Regional Status- In the regional context, Latur, a region in Maharashtra, has a growing interest in aquaculture as an alternative livelihood. Farmers often face challenges like water quality issues and fish diseases, which directly affect productivity. Introducing ZnO NPs as an antimicrobial agent could provide practical solutions to these problems. Moreover, honey production in the region supports the availability of natural resources for nanoparticle synthesis, creating economic opportunities for local farmers.

Latur is also known for its agricultural and beekeeping activities. The use of locally available honey for nanoparticle synthesis can create value-added opportunities for local communities, integrating agriculture, beekeeping, and aquaculture in a sustainable manner.

Scope /Significance of Study- This study focuses on the biosynthesis, characterization, and effects of honey-mediated zinc oxide nanoparticles (ZnO NPs) on the morphology of *Labeo rohita*, a critical freshwater fish in aquaculture. The green synthesis of ZnO NPs using honey, an eco-friendly and sustainable approach, addresses global concerns about chemical pollution in nanoparticle production. The study bridges gaps in understanding the safe application of ZnO NPs, renowned for their antimicrobial properties, in enhancing fish health and disease management.

7. PROPOSED WORK PLAN WITH A TENTATIVE CHAPTER SCHEME :

The work plan of the proposed study can be summarized into following chapters respectively –

1. Introduction.
2. Review of Literature.
3. Material and methods.

4. Synthesis and Characterization of Honey mediated ZnO nanoparticles.
5. Study of antimicrobial activity of ZnO nanoparticles.
6. Specimen collection from study area and physicochemical parameters of Water during Study Period.
7. Morphological and Morphometric analysis of *Labeo rohita*.
8. Biochemical, hematological and histological effects of ZnO NPs on the freshwater fish *Labeo rohita*.
9. Result and discussion.
10. Summary and Conclusion.
11. Bibliography.

8. PROPOSED WORK PLAN/FORMULATION AND STRUCTURE OF STUDY:

First year-

- Collection of literature and references work.
- Standardization of methods and procedures.
- Collection of Honey from study area.

Second year-

- Biosynthesis of Honey mediated Zinc oxide nanoparticles and their characterization.
- Evaluation of Honey mediated ZnO nanoparticles for their antimicrobial activity.
- Specimen collection and physio-chemical parameters.

Third year-

- Morphological and Morphometric analysis of *Labeo rohita*.
- Biochemical, hematological and histological effects of ZnO NPs on the freshwater fish *Labeo rohita*.
- Collection of statistical data and submission of thesis.

9. EXPECTED OUTCOME:

The expected outcome of this study is to successfully synthesize eco-friendly zinc oxide nanoparticles (ZnO NPs) using honey as a natural reducing and stabilizing agent. The nanoparticles are anticipated to exhibit unique physicochemical properties characterized by advanced techniques such as UV-Vis spectroscopy, XRD, and SEM/TEM. When applied to Labeo rohita, these ZnO NPs are expected to influence morphological parameters, including growth, body weight, and fin development. The findings aim to provide insights into the biocompatibility and potential applications of ZnO NPs in aquaculture, emphasizing their role in enhancing fish health while ensuring sustainability through green synthesis methods. The study's outcomes will provide a detailed understanding of the potential benefits and risks of applying ZnO NPs in aquaculture. It will also explore their antimicrobial effects, which may reduce the dependence on antibiotics and synthetic chemicals in fish farming.

The direction of this research work can be adjusted depending upon the findings from time to time, to attain the best expected result within the schedule.

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